The embryo-sac of Aster Novæ-Angliæ.

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WITH PLATES XV AND XVI.

In its early history the ovule of *Aster Novæ-Angliæ* presents little to distinguish it from the well known types of Compositæ. Its anatropous character is already obvious, and its only integument is almost as conspicuous as the nucellus itself, when the archesporium appears. This cell undergoes the expected divisions and, of the four resulting cells, the one farthest from the micropyle is usually, but not always, destined to become the embryo-sac.

Development of the macrospore.

In tracing the early stages in the development of the embryo-sac, I employed methods suggested by Auerbach's researches.[1] He emphasized the significance of the fact that if a preparation containing both spermatozoa and ova be stained with some combination like Biondi-Ehrlich, the nuclei of the spermatozoa take the green while those of the ova prefer the red. He proposed the terms, cyanophilous and erythrophilous to indicate these preferences. Since 1891 a few attempts have been made to obtain analogous results in plants. It is not very difficult to differentiate the nuclei of the pollen grain. The generative nucleus takes the green while the vegetative prefers the red, but I was not able to find a single green nucleus in the embryo-sac. The mother cell of the embryo-sac and also the four cells resulting from its division, stain red. At the first division of the nuclei of the embryo-sac the resulting nuclei stain red. It might be noted, however, that the nucleus which produces the egg apparatus sometimes differs in appearance from the one which gives rise to the antipodal cells. In all the stages which precede fertilization the nuclei stain alike, even the two polar nuclei which unite to form the endosperm-nucleus. Since some have imagined this fusion to be a sexual process, I took particular care in staining, but

[1] AUERBACH, LEOPOLD:—Zur Kenntniss der thierischen Zellen Sitzungsb. der Kgl. preuss. Akademie der Wissenschaften, 26 Juni, 1890, and "Ueber binen sexuellen Gegensatz in der Chromatophilie der Keimsubstantzen," in the same journal of 25 Juni, 1891.

even when the pollen grains and active glands were taking a brilliant green, the fusing nuclei persisted in taking only the red, thus indicating that the fusion has no relation to a sexual process.

With the formation of the endosperm-nucleus the embryo-sac completes its preparations for fertilization. All my figures, except fig. 3, are drawn from embryo-sacs in which the endosperm-nucleus is already formed.

The mature embryo-sac.

The mature sac is surrounded by a beautifully distinct wall of tapetal cells, *t*, figs. 1, 2 and 3, filled withdense protoplasm remarkably free from vacuoles. The egg apparatus usually occupies the micropylar sixth of the sac and displays that reluctance to vary which characterizes structures directly concerned in reproduction. Indeed, there is such uniformity that one knows beforehand just where to focus his mind and microscope to catch the elusive outlines of the synergidæ and micropylar portions of the ovum.

The egg occupies from one-half to four-fifths of the entire diameter of the sac. It well deserves the name, oösphere, for it is often a perfect globe. Usually, however, the egg is pear-shaped, with the smaller end nearest the micropyle; but whatever its shape, there is almost invariably a large vacuole, occupying the greater part of the interior. Below the vacuole in a dense mass of protoplasm, is the egg-nucleus.

The two synergidæ generally fill the entire diameter of the sac. They are somewhat ovate in form and extend from the micropyle to about the middle of the ovum. Their nuclei do not seem to have any favorite position, for they are found, sometimes at one end of the cell, sometimes at the other, but perhaps, more frequently, near the middle. In a few specimens I found the nuclei doubled but never found more than two synergidæ. In two or three cases the principal vacuole was found in the micropylar end, but its usual position is at the opposite extremity. Like the egg, the synergidæ are not protected by any membrane.

As one glances at the mature embryo-sac, its most conspicuous feature is the endosperm-nucleus. Its nucleolus is large, dense and apparently homogeneous, if we except one or more globules which are invariably present. When xylol is used to precede the paraffine, these globules become extremely refractive and seem to be composed of oil. Connect-

ing the nucleolus with the nuclear membrane are delicate radiating filaments. Fig. 2 represents a typical egg-apparatus with its two synergidæ and ovum. The large endosperm nucleus lies just below the egg.

In tabulating measurements of various features of the embryo-sac, I was so impressed by the uniformity in the size of the nucleoli of the egg-apparatus and secondary nucleus that I will give some of the measurements here. The length of the mature sac varies from 250 to 300μ, its diameter at the egg from 35 to 45μ, the diameter of the egg from 22 to 28μ, that of the egg-nucleus from 10 to 13μ, and that of the endosperm-nucleus from 16 to 20μ. The nucleoli, however, presented an unbroken uniformity, the endosperm-nucleolus measuring almost invariably just 10μ, the egg-nucleolus 6μ, which occasionally inceased to 7 or 8μ. The nucleoli of the synergidæ usually measured 4μ, although in a few cases, they reached a diameter of 5 or 6μ.

Development of the antipodal region.

The uniformity which characterizes the egg-apparatus and secondary-nucleus is left behind when we descend to the antipodal region. For this very reason the antipodal cells of *Aster Novæ-Angliæ* furnish an exceptionally interesting field for investigation. The text-books would lead us to expect just seven cells in the mature embryo-sac, and indeed, in the case before us, the sac is often found in this stage, with its egg, two synergidae, secondary-nucleus and three antipodal cells. But previous to the formation of the secondary-nucleus, the antipodal cells frequently enter upon a career of development which can hardly fail to attract attention. My results here do not agree very well with those of Martin,[2] published in this journal. He finds no walls on these cells, finds no cross partitions, never finds more than four antipodal cells and those never arranged in a single longitudinal row. I find that even when there are only three antrpodal cells, they form membranes and are usually arranged in a single longitudinal row; also that when there are more than three antipodal cells, one or more cross partitions are found. From my preparations of Aster and Solidago I should conclude that cell walls, cross partitions and longitudinal arrangement

[2] Development of the flower and embryo-sac of Aster and Solidago. Botanical Gazette 17: 406. D. 1892.

were the rule in these two genera. My sections of embryo-sacs, which were just approaching maturity, resemble Martin's figures and lead me to suspect that his conclusions have been drawn from material in an early stage of development.

Strasburger has noted that there are sometimes more than three antipodal cells, and Mottier[3] figures a case in which each of the three cells has divided.

In rare cases I found just three antipodal cells, each with a single nucleus, three cells with doubled nuclei were not quite so rare, while the condition represented in the figures was not at all uncommon. In fig. 2 we have six antipodal cells, arranged in a single longitudinal row, with the divisions approximately in the same plane. Fig. 11 shows seven antipodal cells which present more complexity in their arrangement. Fig. 13 has nine antipodal cells with still another variation in the plane of division. Fig. 3 goes a step farther and displays thirteen cells. The first three of these cells, of course, arise from free cell formation, but when the number exceeds three, the extra cells are produced by cell division with the formation of partitions. If the partition is not formed at the first division of the nucleus, as in fig. 11, *k*, I am inclined to think that it will not be formed later, at least I have not seen anything which would lead me to believe that partitions are formed after the nuclei have begun to multiply, as in fig. 10.

I am well aware that the doubling of nuclei is not unusual in these cells, but neither my reading nor my preparations of other embryo-sacs foreshadowed the condition represented in fig. 13 where we have thirty nuclei in a single section. Fig. 10 shows twenty nuclei in one section of a single cell. The occurrence of mitotic figures proves that these nuclei multiply by indirect division, but whether they multiply by fragmentation also, I am not prepared to say, although some of the cases figured would suggest such a possibility.

The homology of the antipodal cells has long been a subject for controversy. Without reviewing theories, it seems to me that Strasburger was correct in making them homologous with the prothallium of the gymnosperms. This homology seems sound when we compare their origin with that of the gymnosperm prothallium, but the gymnosperm prothal-

[3] On the embryo-sac and embryo of Senecio aureus L. Botanical Gazette 18: 245. Jl. 1893.

lium proves its title to the name by bearing archegonia. The bearing of archegonia would vindicate the claim of the antipodal cells in the same manner, but my reading has failed to furnish a single instance of such a phenomenon. A glance at my figures will show that the antipodal cells are not all alike, the lower one sometimes differing decidedly from the others. It is often much larger than the rest, it differs in the density of its protoplasm, appearing as if it had increased much more rapidly in size than in substance, and its nuclei resemble the endosperm-nucleus rather than the nuclei of the other antipodal cells. Figs. 1, 2, and 3 illustrate various forms of this cell with its large nuclei. The behavior of this cell recalls the free cell formation which occurs in the early history of the macrospore. This antipodal growth breaks through the layer of tapetal cells which surrounds the embryo-sac, and, continuing its development sometimes to an extent equalling half the original length of the sac, exerts a destructive effect upon the cells of the adjacent tissues. The mere tendency toward further development manifested by the antipodal cells is worthy of careful consideration.

I desire to call particular attention to the lower cell in fig. 3. I feel positive that I have discovered in this cell a veritable oösphere. It has precisely the appearance of the ordinary oöspheres of *Aster Novæ-Angliæ*, even to the position of its nucleus and vacuole and the distribution of its protoplasm. Furthermore, it has no cell membrane, thus differing in another important particular from the usual antipodal cell. It would seem that after the nucleus had divided, one of the daughter-nuclei had surrounded itself with protoplasm and become free, just as the ordinary oösphere originates and separates itself from the surrounding protoplasm of the macrospore. The fact that nuclei of other antipodal cells sometimes surround themselves with protoplasm in a way which recalls the formation of the ordinary oösphere, makes this theory seem possible. Figs. 2 and 6, *x*, furnish examples of such nuclei. It might be suggested that we have here a macrospore, in an unusual position, but a macrospore nevertheless. Whatever its real nature may be, its origin is not so uncertain. In some slides, the septum proves that this cell arises from division; in others, it may be one of the three original antipodal cells. In any case, its origin is not that of the macrospore, but that of the antipodal cell. The ap-

pearance of the accompanying bodies which present such a resemblance to synergidæ and cause a more complete likeness to the egg-apparatus of the other end of the sac, is probably accidental. This figure, like the other figures of the plates, was drawn with an Abbé camera and Zeiss 2^{mm} immersion lens, but the figure fails to show the extent of the resemblance. Lest it might be imagined that I have inverted the embryo-sac and mistaken the endosperm nucleus for the nucleus of this cell, I have drawn in fig. 4, the egg-apparatus of this same sac. It is to be noted here that all this development of the antipodal region has preceded the fusion of the two polar nuclei (fig. 4 *pn*) to form the endosperm-nucleus. It might also be added that the length of this sac is double that of ordinary sacs.

Additional evidence will doubtless be demanded by many, but the frequent occurrence of this peculiar antipodal cell in *Aster Novæ-Anglіæ* leads me to believe that other instances of this phenomenon will be discovered. Indeed, like the unnoticed centrosomes, they may even now be awaiting observation on the slides of earlier investigators. The more conservative may ask that the history of this alleged oösphere be traced at least a few steps further before they allow its right to the name. Let fertilization and the formation of an embryo be observed. It is to be regretted that my material was collected for the purpose of studying the earlier development of the embryo sac rather than the formation of the embryo itself, and consequently the search for another antipodal oösphere, to say nothing of these later stages, is necessarily deferred.

Since reading Strasburger's recent discussion of the periodic reduction of chromosomes,[4] I have been curious to know the number which prevails in the nuclei of these antipodal cells, but as my material was collected late in October, after several severe frosts, mitotic figures were very infrequent and I have not yet been able to obtain any reliable results. Guignard's statement, that in lilies the nucleus which gives rise to the egg-apparatus is constant in its number of chromosomes, while that which produces the antipodal cells varies, is of interest in considering this irregular region.

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[4]On the periodic reduction of chromosomes in living organisms. Annals of Botany **8**: 281. Ag. 1894.

Other Compositæ.

In pursuing these studies, I have made preparations of several other Compositæ, and in Solidago especially I have found considerable irregularity in the number and arrangement of the antipodal cells, but in no other have I found such extensive variation as in *Aster Novæ-Angliæ*.

Methods.

Absolute alcohol, saturated aqueous solution of picric acid, and 1 per cent. chromic acid were used in fixing material for this work. The smaller heads were merely halved; the larger heads were cut into sections about one-eighth of an inch thick before placing in the fixing fluid. Picric acid gave as good results as the chromic. With either acid the fixing is complete in twenty-four hours. Picric material should be washed in 70 per cent. alcohol until all yellow color disappears. Chromic material should be washed at least twenty-four hours in cold water or twelve hours in warm. In either case the water should be changed frequently.

Xylol, followed by a mixture of xylol and paraffine was used to precede the paraffine bath. Rosen's method was also quite satisfactory. After dehydration it is briefly this: (*a*) equal parts of absolute alcohol and bergamot oil; (*b*) pure bergamot oil; (*c*) equal parts of bergamot oil and paraffine at 40° C; (*d*) soft paraffine; (*e*) hard paraffine.

All sections were serial. I did not find it necessary to cut thinner than 5μ.

With the exception of a little material which was stained in bulk with alum carmine, all sections were stained on the slide. Delafield's haematoxylin, acid fuchsin, Bismarck brown and Biondi-Ehrlich are excellent stains for this work. After many slides have been rinsed in the distilled water jar, the water becomes deeply colored. Some slides left over night in this jar to await staining in the morning showed a striking differentiation. My most satisfactory staining was subsequently obtained in this way.

Summary.

1. (*a*) The early development of the macrospore of *Aster Novæ-Angliæ* differs little from described types.
(*b*) The formation of the secondary nucleus has no relation to a sexual process.

2. (*a*) The egg is sometimes spherical and sometimes pear-shaped.
   (*b*) The vacuoles and nuclei of the synergidæ vary in position.
   (*c*) There is a striking uniformity in the size of the nucleoli of the egg-apparatus and the endosperm-nucleus.
3. (*a*) The number of antipodal cells varies from two to thirteen. Six or seven are as frequent numbers as three.
   (*b*) The number of nuclei in an antipodal cell varies from one to over twenty.
   (*c*) The lower antipodal cell differs from the rest in size, density of its protoplasm, appearance of its nuclei, and in its effect upon the surrounding tissues.
4. The discovery of an antipodal oösphere in the antipodal region is an additional proof that the antipodal cells are homologous with the endosperm of the gymnosperms.

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Explanation of Plates XV and XVI.

All figures are drawn from sections of the embryo-sac of *Aster Novæ-Angliæ* and are magnified 407 diameters.

Abbreviations.—*ao*, antipodal oösphere. *en*, endosperm nucleus. *la*, lower antipodal cell. *m*, micropyle. *o*, oösphere. *on*, nucleus of oösphere. *pn*, polar nucleus. *syn*, synergidae. *t*, tapetal cells. *ua*, upper antipodal cell.

Description of figures.—*Figs. 1 and 2.* Sections through the entire embryo-sac. *Fig. 3.* Antipodal cells with an antipodal oösphere. *Fig. 4.* Egg-apparatus of Fig. 3. *Figs. 5–13.* Antipodal region showing variation in number of cells and mode of arrangement. Fig. 8 represents one of the middle antipodal cells.